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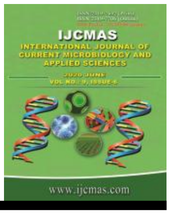

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Identification of Heat Tolerant Hybrids based on Morphological Traits and Selection Indices

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ABSTRACT

Keywords

Maize; Heat stress; Morphological traits; Selection indices; Climate change

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Development of climate resilient cultivars is necessary for maize production as increased temperature have negative impact on maize yield. Selection based on grain yield along with stress-adaptive secondary traits could help in the development of improved, stable heat stress tolerant cultivars. The current study was conducted to identify reliable and effective secondary traits associated with heat stress tolerance in tropical maize. A Six hundred and sixty two double haploid lines derived from nine bi-parental pedigree populations were phenotyped under natural heat stress and optimal condition. Evaluation was carried out under optimal and natural heat stress condition by sowing on 1st week of February and March to expose the reproductive stages and grain filling period during heat stress. Various morphological traits under study were significantly affected by heat stress. ASI found negative correlation and direct effect while EH observed positive correlation and direct effect under heat stress condition. In both conditions, grain yield found positive and significant correlation with STI, MP and GMP as indicated the better heat stress tolerant indices toward Yp and Ys. Results of this study showed that the DH line, DH_1_178 identified as heat tolerant line since this DH line had high grain yield in both conditions and this line can used in future heat tolerant breeding programme.

Introduction

Climate change adversely affects agricultural productivity worldwide. This condition has urged the plant scientists to evolve climate resilient crop varieties, which enable to resist wide spectrum of stresses and help to bring higher production (Kole *et al.*, 2015). Due to climate change, it is predicted that combined heat and drought stress will be major threat to maize crop production and world maize production will lose up to 15-20% per year (Chen *et al.*, 2012; Lobell *et al.*, 2011).

It is predicted that at the end of 21st century, the average increase in temperature will be 2-4°C (Battisti and Naylor 2009). More importantly, predictions based on global climate model analysis suggest that upcoming catastrophe of heat stress will affect the tropical and subtropical regions of the world (Battisti and Naylor 2009). It described that Central and Eastern Asia; Central North America and Northern part of Indian subcontinents will be more liable regions to suffer from heat stress for growing maize and other crops (Teixeira *et al.*, 2013). It is suggested that extreme heat stress at anthesis could potentially decrease 45 per cent of the global maize production by 2080 as compared to 1980 (Deryng *et al.,* 2014).

Nature of heat stress varies widely across the growing seasons and damage to crop is a complex phenomenon being influenced by several factors, thus making it a major concern to physiologists and plant breeders. Plant phenology, developmental phases, growth rates, yield components and final yield of plant are critically affected by thermal regimes. Other than morphological changes, several physiological and biochemical changes (photosynthetic rate, stem sugar mobilization, chlorophyll mobilization) are known to be associated with HS.

Under heat stress, reduction in kernel set was reported (Cantarero *et al.*, 1999; Leipner *et al.*, 1999; Wilhelm *et al.*, 1999; Carcova *et al.*, 2001; Rattalino and Oteguia, 2013), decreased photosynthesis rate (Cantarero *et al.*, 1999; Karim *et al.*, 2000; Ben-Asher *et al.*, 2008), damaged cellular membrane (Ristic *et al.*, 1998) and decreased chlorophyll content (Karim *et al.*, 1997; Havaux and Tardy 1999).

Selection based on grain yield *per se* alone give misleading effects since heritability were normally low under heat stress condition. Previous studies reported that selection based on grain yield along with secondary traits were more reliable in breeding for tolerance under abiotic stresses (Banziger and Lafitte, 1997; Araus *et al.*, 2008; Ober *et al.,* 2005; Ram *et al.,* 2014). The alternative and reliable method of selection based on grain yield along with secondary traits could improve selection response to a large extent since heritability remain high for some secondary traits under stressed environment, and genetically correlated with yield under stress condition. Hence, breeding for stress tolerance could improve by finding key secondary traits that are closely correlated with yield under stressed environment (Ober *et al.*, 2005). Non-significant correlation of traits with grain yield under non-stressed environments while high heritability along with significant correlation with grain yield under stressed environments are some of the necessary criteria for the selection of valuable secondary traits (Bertran *et al.*, 2003). Some different indices (Tolerance Index, Mean Productivity, Geometric Mean Productivity, Stress susceptibility Index and Stress Tolerance Index) had been utilized based on mathematical equations for the selection of stress tolerance genotypes (Fischer *et al.*, 1978; Rosielle *et al.*, 1981; Fernandez, 1992; Gavuzzip *et al.*, 1997). The current study was conducted to find reliable and effective secondary traits associated with heat stress tolerance in tropical maize and identification of heat stress genotypes based on selection indices.

Materials and Methods

Plant materials

A total of six hundred and sixty two DH lines enlisted in Table 1 derived from nine bi-parental pedigree populations, as described below were test-crossed with a heat susceptible tester lines (CML-474) for evaluation under natural heat stress. These test crosses were obtained from CIMMYT, Hyderabad for evaluation under heat stress and optimal conditions at PAU, Ludhiana.

Phenotypic evaluation of DH lines

Field-based assessment of panel was done during spring season 2016 and 2017 at Punjab Agricultural University, Ludhiana. The experiment was conducted in Alpha Lattice design with two replications under normal sown and late sown conditions. Each entry was represented by single row of 3 m length with a spacing of 65 cm between rows and 20 cm between plants. The sowing of one set of trial was delayed (1st week of March) to ensure maximal heat stress during flowering and grain formation during April/May months. Control or normal sowing was done on 1st week of February with same set of lines.

Observation recorded

During the growing season, data was recorded for ten morphological traits including grain yield. Days to 50% anthesis (AD) and silking (SD) were recorded, the date when half of the plants in a plot extruded the first anther and silk in the plot. ASI was calculated as the difference between AD and SD. Leaf firing and tassel blast was recorded as the percentage of plants in a plot with leaf firing and tassel blast symptoms.

Plant height was measured from the soil surface to the base of the tassel (excluding tassel length) and ear height was measured from the soil surface to the base of the ear. The number of ears per plant (EPP) was calculated as the ratio of total number of ears per plot at harvest and total number of plants per plot. Ear position was calculated as the ratio of plant height and ear height. Grain yield was recorded in terms of ear weight per plot immediately after crop harvest and converted to tons per hectare at 12.5% grain moisture content and 80% shelling percentage. Stress tolerance indices were calculated by the following formula, Tolerance Index (TOL) = Yp – Ys (Rosielle and Hamblin 1981), Mean Productivilty (MP) = Ys + Yp/2 (Rosielle and Hamblin 1981), Geometric Mean Productivity (GMP) = $\sqrt{Yp.Ys}$ (Fernandez 1992), Stress Susceptibility Index (SSI) = 1-Ys/Yp/ SI (Fisher and Maurer 1978), in which SI = $= 1 - \overline{Ys} / \overline{Yp}$ and Stress Tolerance Index (STI) = Ys.Yp/ $(\overline{Yp})^2$ (Fernandez 1992), where Ys and Yp being the yield of genotypes evaluated under stress and non- stress condition, $\overline{Ys}$ and $\overline{Yp}$ being the mean of overall genotypes evaluated under stress and non- stress condition.

Phenotypic data analysis

Variance components, σ^2 G, σ^2 GxE and σ^2 e, for the multi-environmental phenotypic data were estimated from analysis of variance (ANOVA) using multi environment trial analysis with R (METAR) (Alvarado *et al.*, 2015). Broad-sense heritability (H^2) of the trails was estimated as:

$$H^2 = \sigma^2 G / \sigma^2 G + \sigma^2 GxE/ l + \sigma^2 e/ l\, r$$

Where, σ^2 G is the genotypic variance, σ^2 GxE is the genotype × environment variance, σ^2 e is the error variance, l is the number of environments, and r is the number of replications. Correlation coefficients between environments and traits, summary statistics (mean, SE, range, LSD, CV) generated using standard procedures implemented in METAR.

Path coefficient analysis was carried out to study the relative importance of direct and indirect effects of secondary traits toward yield. Pearson correlation (phenotypic correlation) was first performed and then the correlation coefficients were generated into direct and indirect effects through path coefficient analysis using Kang SASPATH program developed by Kang (Kang 2015).

Results and Discussion

Phenotypic variation

Significant variability was observed for the traits under study in DH panel under optimal and heat stress conditions. Mean and descriptive statistics of various traits for optimal and heat stress conditions which is presented in Table 2, respectively. The average plant height (175.44 cm) and ear height (80.71 cm) were found in optimal condition is more as there was longer vegetative growth period as compared to heat stress condition. The mean of number of ears per plant was high in optimal condition than heat stress condition. Average grain yield (3.46 t/ha) was low in heat stress environment as compared to optimal environment condition (5.45 t/ha) due to heat stress and less setting under heat stress. These result showed that heat stress highly affected the growth and development of DH lines under study. Similar were also reported by Zaidi *et al.*, (2016); Wang *et al.*, (2016); Alam *et al.*, (2017) and Dao *et al.*, (2017) for reduced grain yield and increased ASI under stress condition. Heat stress has been reported as one of the most important causes of reduction in yield and dry matter production in many crops, including maize (Giaveno and Ferrero, 2003). Broad-sense heritability was calculated for traits under study. Under optimal condition, heritability found high as compared to heat environment condition. This showed that environment play vital role for the expressions of the traits under evaluation. In the present study, genetic variance and heritability were low under heat stress as compared to the optimal condition (Table 2). Similarly, Dixit *et al.*, (2014) and Ramya *et al.*, (2016) had reported declined for genetic variance and heritability under stress. Messmer *et al.*, (2009) and Almeida *et al.*, (2013) also reported for reduction of genetic variance under moisture stress conditions.

Path coefficient analysis and identification of suitable secondary traits

Correlation coefficients of direct and indirect effects towards grain yield under optimal and heat stress conditions were presented in Tables 3 and 4. Under optimal condition, traits *viz.*, AD and ASI showed negative direct effect and negative correlation with grain yield. The correlation represented the true relationship between the traits. Therefore, days to 50% anthesis and anthesis-silking interval indicated a reliable trait for indirect selection for improved grain yield. This result is in agreement with Badu-Apraku *et al.*, (2012), Chapman *et al.*, (1999), Messmer *et al.*, (2009) and Almeida *et al.*, (2009). Similarly, anthesis-silking interval showed negative direct effect and negative correlation with grain yield under heat stress. Days to 50 % silking had low and positive (0.092) direct effect on grain yield but had negative and high indirect effects through AD and ASI may have negative correlation between the two traits ($r= -0.32^{***}$) under optimal condition. The traits such as plant height, ear height and ear position had positive direct effect on grain yield and their association with grain yield were also significant and positive, indicated the true and perfect relationship between grain yield and these characters under optimal condition. Hence, suggesting direct selection based on these characters would help in selecting the high yield genotypes in maize. Similarly, in heat stress condition, canopy temperature and ear height had positive direct effects on grain yield and positive significant correlation with grain yield. EPO found negative (-0.294) direct effect on grain yield but the positive and high indirect through ear height caused the positive correlation (0.167) under stress condition. Selection of EPO can be considered via ear height.

In the present study, genetic variability for grain yield and associated secondary traits was observed among the DH lines. This variability will be helpful for selection of heat tolerance DH lines with high yield potential, either by drought escape mechanisms or by using secondary traits in a selection index. Araus *et al.,* (2008) suggested that the relative value of secondary traits for indirect selection for grain yield is determined based on variance, heritability and genetic correlation with yield. Hence, ASI was identified as most important indirect selection criteria with grain yield to use in the selection for developing heat tolerance lines.

Selection of DH lines based on heat stress tolerance indices

Correlation coefficient analysis between Yp, Ys and different indices of stress tolerance were calculated to determine the most desirable criterion (Table 5). There were significant positive correlation between Yp with TOL, STI, MP, SSI and GMP. Similarly, there were significant positive association between Ys with STI, MP and GMP while TOL and SSI found negative non-significant. It is also observed significant positive association between the heat stress tolerance indices except between STI and GMP with SSI. GMP, MP and STI consequently appeared as better predictors of Yp and Ys as compared to TOL and SSI, respectively. This result is in agreement with Khodarahmpour *et al.,* (2011) finding.

Among the DH lines, DH_1_13 (4.58 t/ha), DH_3_126 (4.56 t/ha) and DH_4_23 (4.55 t/ha) produced higher grain yield under heat stress condition while DH_1_199 (6.87 t/ha), DH_1_53 (6.82 t/ha), DH_1_178 (6.76 t/ha) produced higher grain yield under optimal condition (Table 6). Based on SSI and TOL, DH_4_23, DH_9_9, DH_9_107 and DH_9_98 identified as tolerant DH lines (Table 6). Low TOL does not means high yielding, hence, genotypes yield should be consider in addition to TOL criterion. Similarly, based on STI, MP and GMP, DH_1_178 found highest heat tolerance and recorded high grain under optimal (6.76 t/ha) and heat stress (4.17 t/ha) condition (Table 6).

Table.1 List of material used in the present investigation for evaluation under heat stress conditions

S.No.	Population code	Pedigree	No. of DH lines
1	YCMLZH1393	VL109524/VL1036	62
2	YCMLZH1383887	ZL152847/ZL152840	82
3	YCMLZH111666	VL1030/VL1055	62
4	YCMLZH111497	VL108869/VL1036	54
5	YCMLZH111500	VL062605/VL1036	81
6	YCMLZH1378	VL1018114/VL1036	87
7	YCMLZH1369	VL1018146/VL1036	65
8	YCMLZH138386	VL1033/VL105611	87
9	YCMLZH1383888	VL1110201/VL1110232	82
		TOTAL	662

Table.2 Mean and descriptive statistics of traits under optimal and heat stress conditions

Condition	Trait	Mean	Max	Min	LSD	CV	σ^2 G	σ^2 GE	H^2
Optimal	GY	5.45	6.96	3.15	2.62	24.59	0.55***	0.16***	0.51
	AD	71.17	78.81	66.99	4.00	2.87	3.87***	0.29***	0.77
	SD	72.83	83.31	68.51	4.45	3.11	4.57***	0.39***	0.76
	ASI	1.78	3.60	0.62	3.20	92.10	0.69***	0.18***	0.51
	PH	175.44	191.51	151.56	28.44	8.27	61.93***	6.17***	0.53
	EH	80.71	70.93	91.84	23.19	14.66	27.87***	10.29***	0.41
	EPO	0.46	0.52	0.45	0.19	14.66	0.0004	.0002	0.41
Heat stress	GY	3.46	4.58	2.55	2.57	37.93	0.35***	0.25***	0.38
	SD	71.68	72.62	71.24	7.07	5.03	0.55***	7.45***	0.07
	ASI	2.69	5.63	0.25	4.45	84.51	1.07***	0.35***	0.42
	PH	149.13	153.13	144.07	29.04	9.93	15.14***	42.45***	0.17
	EH	62.74	69.53	55.41	22.03	17.92	18.11***	16.89***	0.31
	EPO	0.42	0.47	0.38	0.11	13.54	0.0004	.0002	0.32

*= 0.05% significant, **= 0.01% significant, ***=0.001% significant, σ^2 G= Genotype variance, σ^2 GE= Genotype X Environment variance, Max=Maximum, Min= Minimum, H^2 = broad-sense heritability, LSD= Least significant difference at 5% level, GY= Grain yield, AD= Days to 50% anthesis, SD= Days to 50% silking, ASI= Anthesis-silking interval, PH= Plant height, EH= Ear height and EPO= Ear position

Table.3 Direct (Diagonal) and indirect (above and below diagonal) path effects of different characters toward grain yield under optimal condition

	AD	SD	ASI	PH	EH	EPO	R
AD	**-0.280**	0.085	-0.070	0.02	0.006	-0.0005	-0.24***
SD	-0.258	**0.092**	-0.148	-0.003	-0.001	-0.001	-0.32***
ASI	-0.070	0.049	**-0.280**	-0.094	-0.023	-0.001	-0.42***
PH	-0.017	-0.001	0.078	**0.337**	0.080	0.003	0.48***
EH	-0.017	-0.001	0.062	0.256	**0.105**	0.015	0.42***
EPO	0.006	-0.004	0.014	0.030	0.054	**0.029**	0.13**

*= 0.05% significant level, **= 0.01% significant level, ***=0.001% significant level, r = Coefficient of phenotypic correlation with grain yield, GY= Grain yield, AD= Days to 50% anthesis, SD= Days to 50% silking, ASI= Anthesis-silking interval, PH= Plant height, EH= Ear height and EPO= Ear position

Table.4 Direct (Diagonal) and indirect (above and below diagonal) path effects of different characters toward grain yield under heat stress condition

	ASI	CT	EH	EPO	R
ASI	**-0.410**	-0.051	-0.082	0.054	-0.490***
CT	0.124	**0.169**	0.137	-0.100	0.331***
EH	0.089	0.061	**0.381**	-0.292	0.279***
EPO	0.076	0.057	0.327	**-0.294**	0.167***

*= 0.05% significant level, **= 0.01% significant level, ***=0.001% significantlevel, r = Coefficient of phenotypic correlation with grain yield, GY= Grain yield, ASI= Anthesis-silking interval, CT= Canopy temperature, EH= Ear height and EPO= Ear position

Table.5 Correlation coefficients between grain yield and stress selection indices

	Yp	**Ys**	**TOL**	**STI**	**SSI**	**MP**
Ys	0.331***					
TOL	0.222***	-0.006				
STI	0.798***	0.830***	0.130***			
SSI	0.122**	-0.075	0.957***	0.024		
MP	0.333***	0.319***	0.393***	0.411***	0.155***	
GMP	0.311***	0.339***	0.249***	0.411***	0.009	0.988***

*=0.05% significant, **=0.01% significant, ***=0.001% significant, Yp= Grain yield under optimal condition, Ys= Grain yield under heat stress condition, TOL= Stress tolerance, STI= Stress tolerance index, SSI= Stress susceptibility, MP= Mean productivity and GMP= Geometric mean productivity

Table.6 Mean comparison related to DH lines yield under optimal condition (Yp), heat stress condition (Ys), Tolerance Index (TOL), Mean Productivity (MP), Stress Susceptibility Index (SSI), Stress Tolerance Index (STI) and Geometric Mean Productivity (GMP)

DH line	**Ys**	**Yp**	**SSI**	**TOL**	**MP**	**STI**	**GMP**
DH_1_13	4.583	5.943	0.634	1.359	5.263	0.771	5.219
DH_3_126	4.561	5.438	0.447	2.736	4.582	0.540	4.373
DH_4_23	4.558	4.331	-0.146	1.605	4.388	0.691	4.314
DH_1_199	3.724	6.871	1.269	3.148	5.297	0.542	5.058
DH_1_53	4.053	6.828	1.126	2.775	5.440	0.594	5.260
DH_1_178	4.173	6.767	1.061	2.591	5.468	0.617	5.314
DH_4_23	4.558	4.331	-0.146	1.605	4.388	0.691	4.314
DH_9_9	3.175	3.194	0.016	1.815	4.287	0.651	4.189
DH_9_107	3.274	3.456	0.146	2.037	4.610	0.638	4.496
DH_9_98	3.150	3.373	0.183	1.899	4.072	0.622	3.959
DH_1_178	4.173	6.764	1.061	2.591	5.468	0.616	5.313

Genotypic variability for heat stress tolerance exists in DH lines, which is prerequisite for selection. An understanding of genetic effects involved in inheritance of various morphological and physiological parameters controlling heat tolerance in the genetic material should be thoroughly researched to divert efforts in that direction and to formulate effective selection criteria. Among the secondary traits, ASI along with grain yield found effective for indirect selection to develop heat stress tolerance. DH_1_178 found more heat tolerance and recorded high grain under optimal and heat stress condition.

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